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EFFECTIVENESS OF THE 2003–2004 INFLUENZA VACCINE AMONG U.S. MILITARY BASIC TRAINEES: A YEAR OF SUBOPTIMAL MATCH BETWEEN VACCINE AND CIRCULATING STRAIN

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Effectiveness of the 2003–2004 influenza vaccine among U.S. military basic trainees: a year of suboptimal match between vaccine and circulating strain☆

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Abstract

Effectiveness of the 2003–2004 influenza vaccine was evaluated at five military basic training centers throughout the United States. Data from surveillance conducted in December and January 2003–2004 in this highly vaccinated population were evaluated. During this period, 10.6% (37/350) of specimens were positive for influenza A. A 14-day period after vaccination was considered the period prior to immune protection; vaccine effectiveness (VE) was calculated based on febrile respiratory illness presentation and laboratory confirmation of influenza before or after this 14-day period. Thirty-two cases presented within 14 days of vaccination, and five cases presented beyond 14 days from vaccination. VE in this population was estimated to be 94.4% for laboratory-confirmed influenza. In contrast, VE was only 13.9% for influenza-like illness (ILI) without a laboratory confirmation.
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Keywords: Influenza; Vaccine effectiveness; Military recruits; Vaccine mismatch

1. Introduction

With the influenza virus' unique ability for genetic recombination and drift, the influenza vaccine must be reformulated annually to cover anticipated circulating strains. The 2002–2003 influenza season saw emergence of a drifted, antigenically distinct H3N2 characterized as A/Fujian/411/2002. Early attempts to grow this variant in eggs for inclusion in the 2003–2004 northern hemisphere influenza formulation were unsuccessful, however [1]. Therefore, the influenza vaccine formulation for the 2003–2004 season remained unchanged, with three inactivated viral components, one of which was the H3N2 influenza A virus, A/Panama/2007/99.

As predicted, early in the 2003–2004 season the predominant circulating strain was noted to be the influenza A/Fujian/411/2002 (H3N2) variant. There was concern that the vaccine would not provide protection against this strain, although partial protection, leading to an attenuated illness, was expected. Early reports of clinical effectiveness, however, were quite concerning. The season's influenza vaccine was estimated to be only 3–14% effective against influenza-like illness (ILI) in one report, but its effectiveness against laboratory-confirmed influenza was not measured [2]. Recently, this report was augmented with an adult case–control

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study of laboratory-confirmed influenza cases and random matched controls, and reported a higher vaccine effectiveness of 38–52% [3]. Other recent analyses from this past season suggested vaccine coverage from 25 to 69% [4–6]. Historically, influenza vaccine effectiveness was measured among Finnish military recruits in 1997 during a similar period of vaccine and circulating strain mismatch. In this study, the influenza vaccine was 57% effective in preventing laboratory diagnosed influenza infections [7].

The Naval Health Research Center (NHRC) has conducted surveillance in military recruit training centers for febrile respiratory illness since 1996, hereafter referred to as influenza-like illness [8]. Laboratory testing of samples obtained was expanded to include influenza, among other viral pathogens, early in 1998. During the winter season, influenza vaccination (inactivated vaccine formulation) is required for members of the military; this requirement is stringently enforced at recruit training centers. Data collected this influenza season included date of receipt of the influenza vaccination. It became apparent early in the season that the vast majority of individuals with a laboratory diagnosis of influenza had only recently received the vaccine, whereas influenza-positive results from individuals who had received the vaccine some time prior were rarely encountered.

For the entire season at all surveyed training sites, 120 cases presented with no vaccination or within 14 days of vaccination, but only 15 cases presented greater than 14 days after vaccination. The magnitude of this observation was startling, but potentially could be explained by rapid, early transmission of influenza in a population that was only recently vaccinated, with few vulnerable to infection with earlier vaccination. To evaluate this perceived protection conferred by influenza vaccination during the 2003–2004 season, an epidemiologically sound methodology was pursued for estimation of vaccine effectiveness in military recruit populations.

2. Materials and methods

2.1. Capture of influenza-like illnesses

Surveillance for ILI among recruits was first initiated by NHRC in 1996. Expanded in 1998, the network now includes eight recruit training centers throughout the United States five of these had influenza transmission during December and January and were included in this analysis. A case of ILI is defined as an individual presenting for medical care with an oral temperature $\geq$38 °C (100.5 °F), plus a cough and/or sore throat. On-site dedicated staff capture numerator data (individuals meeting case definition) and denominator data (total recruit population at each site), and rates are calculated. A selection of those meeting the case definition is sampled with a throat swab, and a questionnaire completed, which includes date of recent influenza vaccination. Samples are stored at −70 °C, and forwarded to the NHRC Respiratory Disease Laboratory on dry ice for viral culture and molecular diagnostic processing.

Table 1
Universal and H3-specific primers used in the molecular identification of influenza from original patient specimens

Primer set 1: amplicon size 243 bp	
FluAUniversal1NHRC_F	5′ GAC CIA TCC TGT CAC CTC TGA C 3′
FluAUniversal1NHRC_R	5′ CAT ICA ACT GGC IAG IGC AC 3′
Primer set 2: amplicon size 93 bp	
FluAUniversal2LD_F	5′ AGC AAA AGC AGG TAG ATR TT 3′
FluAUniversal2LD_R	5′ TCG GCT TTG AGG GG 3′
Primer set 3: amplicon size 1174 bp	
Flu A_H3_7F	5′ ACT ATC ATT GCT TTG AGC 3′
Flu A_H3_1184R	5′ ATG GCT GCT TGA GTG CTT 3′

bp, base pair.

2.2. Laboratory processing

All samples received were processed for viral isolation in rhesus monkey kidney cells (Diagnostic Hybrids, Athens, OH). Identification of infecting viruses was performed with fluorescein-labeled monoclonal antibodies (IFA) after cytopathic effect was noted [9].

For molecular detection of influenza, DNA and RNA were extracted from the original patient specimens using the MasterPure™ Complete DNA and RNA Purification Kit (Epicentre, Madison, WI) according to the manufacturer's instructions. Reverse transcription-polymerase chain reaction (RT-PCR) using two primer sets was used to identify influenza A-positive samples (Table 1). Amplification reactions for the RT-PCR were carried out using the OneStep RT-PCR kit (Qiagen) according to manufacturer's instructions, modified for a final concentration of 1× Q solution and a final reaction volume of 25 μl. Two microliters of RNA template was used. Each reaction was subjected to one cycle of reverse transcription (45 min at 50 °C), one cycle of RT denaturation and hot-start activation (95 °C for 15 min), 35 cycles of amplification (primer set 1: 94 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min; primer set 2: 94 °C for 30 s, 50 °C for 1 min, 72 °C for 1 min), and one final cycle at 72 °C for 10 min. Products were analyzed using gel electrophoresis with ethidium. Samples were considered "influenza positive" if inoculated cell cultures were positive by IFA, or if both primer set 1 and primer set 2 yielded positive results.

An 1174 base-pair segment of the hemagglutinin gene was sequenced from a selection of influenza A-positive samples from all surveillance sites. Primer set 3 (Table 1) was used with the BigDye® Terminator v3.1 according to the manufacturer's instructions. Samples were run on the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Sequence analysis was done using DNASTAR software and sequences were compared with published sequences in GenBank®. Sequences were identified as being genetically related to the Fujian/411 lineage if they contained amino

Table 2
Vaccine effectiveness calculated using only laboratory-confirmed influenza, and using the assumption of 14 days required after vaccination prior to considering one 'protected by vaccine'

Site	Vaccinated person–weeks	Unvaccinated person–weeks	Cases vaccinated	Cases unvaccinated	Calculated vaccine effectiveness (%)
1	35,715	11,905	5	11	84.8
2	37,690	12,563	0	4	100.0
3	19,609	3931	0	5	100.0
4	36,885	18,415	0	10	100.0
5	2632	877	0	2	100.0
Total	132,531	47,691	5	32	94.4

acid substitutions at H155T and Q156H, and were phylogenetically more closely related to the Fujian strain than the Panama.

2.3. Calculation of vaccine efficacy

Recruits receive mandatory influenza vaccination during the influenza season at all sites upon arrival. For this analysis, an individual was considered protected by vaccination 14 days after receiving the vaccine. Although this is a commonly accepted time period before immune system response to vaccine is considered complete, a 7-day lag period was also used in an alternative analysis. In October and November, all recruits who arrived for recruit training were vaccinated, but those recruits already present were gradually captured until all recruits on site were vaccinated. By December 2003, queries at each recruit training center confirmed that coverage was essentially 100%. For this reason, only recruits encountered in December and January were included in this analysis, since accurate assumptions on the percentage of the population susceptible could not be made before this time.

For the purposes of person–time contributions in the vaccine effectiveness calculations, 25% of recruits in 8-week training programs (14 days/8 weeks) were considered not protected by vaccination, pending development of immunity. Likewise, at a 6-week program, 33% (2/6) were considered not protected, and at a 12-week program, 17% (2/12) were considered not protected by vaccination. Total person–weeks in recruit training for these months were acquired directly from the training centers. VE was calculated as: $100 \times [1 - \text{Relative risk} = 1 - (\text{rate in vaccinated group}) / (\text{rate in unvaccinated group})]$.

3. Results

Among all ILI specimens collected during this period from these five military basic training centers, 10.6% (37/350) were positive for influenza A virus, either by culture or molecular techniques. Hemagglutinin gene sequencing was performed on 20 of the 37 isolates, representing isolates from all five sites. All 20 isolates carried the characteristic amino acid changes of the Fujian/411 strain. Similarly, of 918 U.S. influenza isolates antigenically characterized by the Centers for Disease Control and Prevention in the 2003–2004 influenza season, 88.5% were noted to be this drift variant [10].

Table 2 presents data for laboratory-confirmed influenzas, and Table 3 presents rates of individuals presenting with ILI, without laboratory confirmation. VE of 94.4 and 13.9% are seen, respectively. By modifying the assumption of time to coverage from 14 to 7 days, the calculated VE changed only slightly from 94.4 to 94.3% (data shown only for 14-day calculation).

It can be seen in Table 2 that all five cases of influenza from vaccinated individuals came from the same site. These cases clustered spatially within a 2-week period, but occurred in different units of the recruit camp. A phylogenetic comparison was performed to determine if these isolates differed from influenza isolates acquired from unvaccinated individuals, thus leading to vaccine evasion. A 299 amino acid segment from the open reading frame of the HA1 hemagglutinin gene was compared in all 20 sequenced samples (four from vaccinated recruits, 16 from unvaccinated); differences were randomly distributed between the two groups, suggesting that vaccine break-through with these recruits was not the result of further drift of the influenza virus.

Table 3
Vaccine effectiveness calculated using all influenza-like illness 'without' laboratory confirmation as influenza, and using the assumption of 14 days required after vaccination prior to considering one 'protected by vaccine'

Site	Vaccinated person–weeks	Unvaccinated person–weeks	Cases vaccinated	Cases unvaccinated	Calculated vaccine effectiveness (%)
1	35,715	11,905	54	24	25.0
2	37,690	12,563	44	25	41.3
3	19,609	3931	52	11	5.2
4	36,885	18,415	53	25	−5.8
5	2632	877	17	7	19.0
Total	132,531	47,691	220	92	13.9

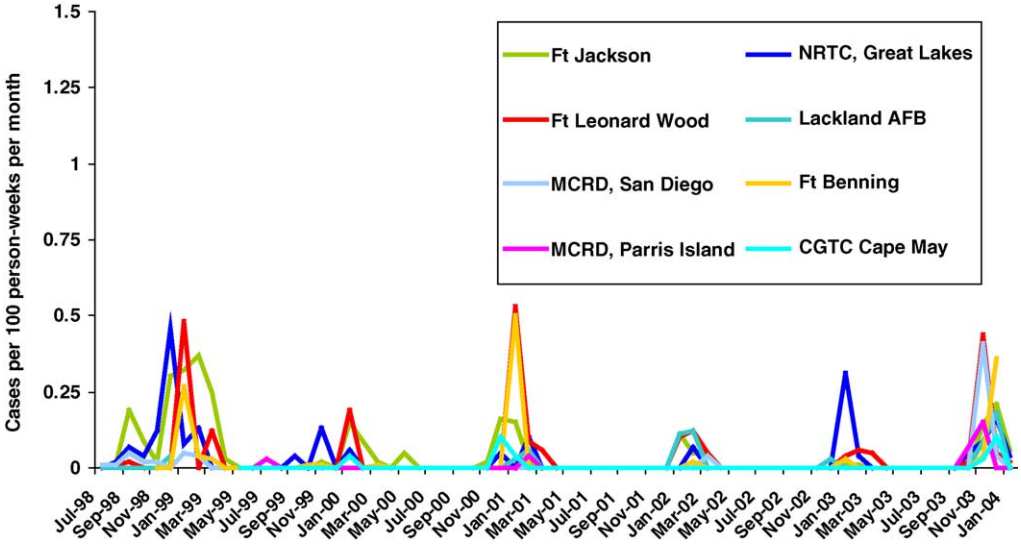

Fig. 1. Rates of influenza illness among U.S. military recruits at eight training centers. The 2003–2004 season was similar to other recent years in burden of influenza illness in this highly vaccinated population.

Fig. 1 demonstrates rates of laboratory-confirmed influenza illness over the past 6 years. As can be seen, this past season was quite comparable to previous years, where no concern over mismatch of the vaccine to circulating influenza strains existed. Observing these rates of influenza in this highly vaccinated population again suggests that this season's influenza vaccine provided reasonable coverage.

4. Discussion

These estimates of vaccine efficacy are more reassuring than previous ones offered in the 2003–2004 influenza season, despite the fact that Fujian/411-like strains of influenza virus were clearly circulating at recruit training centers during this season. These analyses are strengthened by using laboratory-confirmed influenza cases, rather than only reported ILI without laboratory confirmation.

One might hypothesize that recruits with less severe illness are less likely to present for medical care. Indeed, presentation for medical attention is required for capture in our surveillance network. Recruits might hesitate to report for medical attention for fear of being pulled out of training or "set-back" or "recycled" in training; therefore, recruits may delay medical treatment if they are able to endure milder symptoms. Using this logic, recruits with more severe illness would be more likely to seek medical care, and our surveillance would disproportionately capture these more severe illnesses, thus biasing results toward noting a more robust vaccine effect. These findings, therefore, may reflect that vaccination resulted in attenuation of illness, rather than complete protection. One might also hypothesize that the further recruits have progressed in training, the less likely they would want to jeopardize their hard work, and present for medical care. This, likewise, would bias toward an inflated VE.

This analysis suggests that the 2003–2004 influenza vaccine formulation was highly effective in preventing laboratory-confirmed influenza illness in young military recruits. Despite potential limitations in study design and analysis, this evaluation remains important. Military populations are highly vaccinated and provide critical information regarding effectiveness of current influenza vaccine formulations. Additionally, this methodology can easily be repeated year after year, providing relative effectiveness of the current influenza vaccine formulation as compared with previous years. Influenza vaccination, by its very nature, must be evaluated annually for evidence of mismatch between circulating strains and coverage provided by the current formulation.

The U.S. military has a critical need to know the real-time risk of influenza among its service members. Military readiness is enhanced as we continue to monitor the effectiveness of prevention regimens, including vaccination efforts. The Department of Defense efforts thereby supplement civilian public health surveillance efforts. Given the alarming potential for eliciting worldwide pandemics, consistent and rigorous surveillance for influenza must remain a priority.

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